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13. ABSTRACT ***(Maximum 200 Words)***

Our studies investigate the *in vivo* effects of soy isoflavones on human breast tissues obtained from lumpectomy/mastectomy specimens. We investigate the effect of increased tissue concentration of isoflavones for a period of three weeks on breast cell proliferation, differentiation and cell cycle regulatory proteins. Patients with ductal carcinoma *in situ* (DCIS) or invasive breast cancer are randomly assigned to take 100 mg soy isoflavone (Novasoy™, Archer Daniels Midland Company, Decatur, Illinois) or placebo daily for three weeks prior to surgery. Plasma isoflavone levels are measured at baseline and after three weeks in both groups. Tissue isoflavone levels are measured on samples from benign breast tissues in both groups. Biomarker studies are performed on surgical specimens by immunohistochemistry and Western blot. These studies will enable us to determine if a short duration of exposure to increased tissue levels of isoflavones will modulate biomarkers of cell differentiation (Cx43), adhesion (E-cadherin), proliferation (MIB-1), and cell growth and apoptosis (bcl-2, bax, p53, p21, Rb, EGF-R, cyclin D1, CDK5, CDK6) in benign, pre-malignant and malignant areas of breast epithelial tissues. Biomarker studies on the patients randomized on this study will be completed in early 2003.

We have conducted a pilot study in six women, who took soy isoflavones 50 mg (Novasoy™) daily for three weeks. DNA was isolated from the nuclei of peripheral blood lymphocytes and analyzed for levels of 5-hydroxy-methyl-2'-deoxyuridine (5-OHmdU) by gas chromatography-mass spectrometry. The mean level of 5-OHmdU was decreased by 35% (relative to baseline) after 1 week and by about 50% after 2 weeks and 3 weeks of supplementation. Mean plasma levels of 8-isoprostanes also decreased somewhat after supplementation.

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3. TABLE OF CONTENTS

4. INTRODUCTION

Epidemiological studies have shown an inverse association between dietary intake of fruits and vegetables and carcinoma of the breast. One group of major micronutrients in vegetables and fruits, which have been postulated to prevent breast cancer, are soy isoflavones. The mechanism by which isoflavones may prevent breast cancer is not known. Based on our preliminary studies, we hypothesize that isoflavones inhibit cell proliferation, upregulate the expression of gap junctional protein connexin 43 (cx43) and alter the expression of cell cycle regulatory proteins. Our studies will investigate the *in vivo* effects of isoflavones on human breast tissues obtained from lumpectomy/mastectomy specimens. We will investigate the effect of increased tissue concentration of isoflavones for a period of three weeks on breast cell proliferation, differentiation and cell cycle regulatory proteins. Sixty-four patients with ductal carcinoma *in situ* (DCIS) or invasive breast cancer scheduled to have surgery will be randomly assigned to supplement their diet with 100 mg soy isoflavone or placebo daily for three weeks. Plasma isoflavone levels will be measured at baseline and after three weeks in both groups. Tissue isoflavone levels will be measured on samples from surgical specimens, in benign and malignant areas of the epithelia, in both groups. Biomarker studies will be done on surgical specimens by immunohistochemistry and Western blot analysis. Comparisons will be made between areas of comparable microscopic characteristics [malignant, DCIS, lobular carcinoma *in situ* (LCIS), dysplasia, hyperplasia and benign] on breast tissues of patients from intervention and control groups. These studies will enable us to determine if a short duration of exposure to increased tissue levels of isoflavones will modulate biomarkers of cell differentiation (cx43), adhesion (E-cadherin), proliferation (MIB-1), and cell growth and apoptosis (bcl-2, bax, p53, p21, Rb, EGF-R, cyclin D1, CDK5, CDK6) in benign, pre-malignant and malignant areas of breast epithelial tissues. In addition, since baseline biopsy samples are available, a limited number of the marker studies (prioritized in the order cx43, bcl-2, p21, CDK5) will be performed on pre-intervention biopsy samples of patients in the intervention group, giving us an opportunity to compare pre- and post-intervention marker levels in the same patient.

5. BODY

During the first year of the study, there was a delay in getting the study started because of difficulty in hiring study personnel and change of study personnel. An additional delay in starting the study was due to changes made in the study design by introducing isoflavone and placebo tablets and making patients with invasive cancer also eligible for entry. The study intervention was changed from soy protein isolate to soy isoflavone tablet, in order to make the study intervention easier to take and to improve the compliance with the study intervention. The change also improved the study design by introducing a placebo arm instead of a no intervention arm. The study design is now better scientifically and it is easier for the patients to accept a placebo controlled study compared to one with no intervention arm. However, these changes resulted in additional delays in starting the study because of resubmission to the IRB.

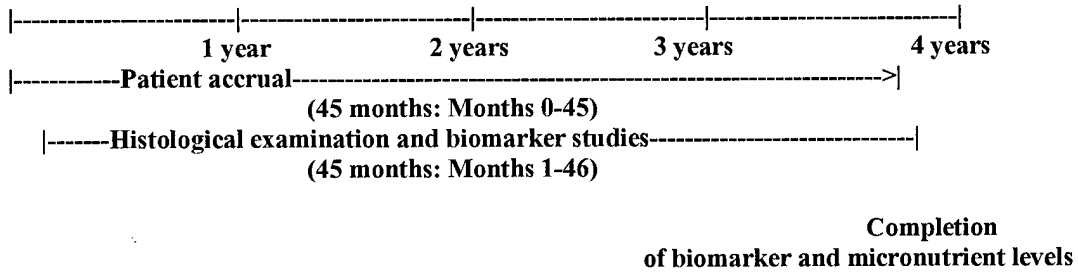
The study is currently accruing at a rate of 1.75 patients per month, which is sufficient to complete the study in accordance with the objectives stated in the grant application. In our

proposal the predicted accrual rate was 1.6 subjects per month. We randomized 21 patients during the first 12-months of the study, which gives an accrual rate of 1.75/month. However, since the study did not start until August 1999, the accrual will continue until we have 72 subjects randomized. We project from the accrual data that the accrual will be completed in 28 months and final analysis will require another 3 months. Therefore, the target date of completion of all study goals is estimated to be 31 months from October 1, 2000, i.e. May 1, 2003. This will require a no-cost extension of the study by 7 months. We will request this extension, if necessary, before the end of the grant period, because increased accrual over the next two years may make the extension unnecessary.

Below please find the Statement of Work copied from the original application, which outlines the work to be completed over the period of the grant.

STATEMENT OF WORK

Timeline:



It is estimated that over a period of 45 months, 64 eligible and evaluable patients will be randomized on the study. We will evaluate study compliance by returned remaining pill counts and questioning the subjects with regard to their intake of study or non-study soy supplements. Since the duration of study is only three weeks and soy isoflavone is a non-toxic nutritional supplement, non-compliance with the study intervention should not be a problem in this study. However, to accomodate a non-compliance rate of about 4 patients on each arm we will randomize a total of 72 patients over 45 months, i.e. an accrual rate of 1.6 patient/month.

During the last three months of the study, we will complete the final three-week intervention/non-intervention period and surgery. This will leave approximately 9-10 weeks for completion of the biomarker and micronutrient analyses on the most recent patients and overall biostatistical analyses of the results. Biomarker studies will be performed throughout the grant period between the first month and the 46th month.

6. KEY RESEARCH ACCOMPLISHMENTS

As the study is double blind, randomized in design, no findings can be attributed to study intervention until the end of the study period when the code will be broken and the identity of the study compounds taken by the patients will be known.

7. REPORTABLE OUTCOMES

Because of the study design, again, no reportable outcomes can be provided until the end of the study period when the code will be broken and the identity of the study compounds taken by the patients will be known.

However, we reported the results of a pilot study with a poster presentation at the 2000 DOD Era of Hope Meeting in Atlanta in June 2000. This study was conducted as a part of this application. Please find below the abstract presented at the DOD Meeting.

EFFECTS OF SOY ISOFLAVONES ON BIOMARKERS OF OXIDATIVE STRESS AND CELL GROWTH IN PATIENTS WITH BREAST CANCER

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Ecological studies have shown an inverse association between dietary intake of soy isoflavones and development of breast cancer. The mechanism by which soy isoflavones may prevent breast cancer is not known. Based on our preliminary studies, we hypothesize that soy isoflavones inhibit cell proliferation, upregulate the expression of gap junctional protein connexin 43 (Cx43) and alter the expression of cell cycle regulatory proteins. Our studies investigate the *in vivo* effects of soy isoflavones on human breast tissues obtained from lumpectomy/mastectomy specimens. We investigate the effect of increased tissue concentration of isoflavones for a period of three weeks on breast cell proliferation, differentiation and cell cycle regulatory proteins. Patients with ductal carcinoma *in situ* (DCIS) or invasive breast cancer are randomly assigned to take 100 mg soy isoflavone (Novasoy^{TM}, Archer Daniels Midland Company, Decatur, Illinois) or placebo daily for three weeks prior to surgery. Plasma isoflavone levels are measured at baseline and after three weeks in both groups. Tissue isoflavone levels are measured on samples from benign breast tissues in both groups. Biomarker studies are performed on surgical specimens by immunohistochemistry and Western blot. These studies will enable us to determine if a short duration of exposure to increased tissue levels of isoflavones will modulate biomarkers of cell differentiation (Cx43), adhesion (E-cadherin), proliferation (MIB-1), and cell growth and apoptosis (bcl-2, bax, p53, p21, Rb, EGF-R, cyclin D1, CDK5, CDK6) in

benign, pre-malignant and malignant areas of breast epithelial tissues. Biomarker studies on the patients randomized on this study will be completed in early 2003.

We have conducted a pilot study in six women, who took soy isoflavones 50 mg (Novasoy™) daily for three weeks. DNA was isolated from the nuclei of peripheral blood lymphocytes and analyzed for levels of 5-hydroxy-methyl-2'-deoxyuridine (5-OHmdU) by gas chromatography-mass spectrometry. The mean level of 5-OHmdU was decreased by 35% (relative to baseline) after 1 week and by about 50% after 2 weeks and 3 weeks of supplementation. Mean plasma levels of 8-isoprostanes also decreased somewhat after supplementation.

8. CONCLUSIONS

Because of the study design, again, no conclusionss can be drawn until the end of the study period when the code will be broken and the identity of the study compounds taken by the patients will be known.

9. REFERENCES

Since the study is still accruing patients, no publications can be reported until the end of the study when the code will be broken and the identity of the study compounds taken by the patients will be known.

10. APPENDICES

No appendices are available for submission at this time.